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Bioenergy potential of *Chlorella vulgaris* under the influence of different light conditions in a bubble column photobioreactor

S. Dhanasekar, R. Sathyanathan*

Department of Civil Engineering, Faculty of Engineering and Technology, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu, Tamil Nadu 603203, India

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ABSTRACT

BACKGROUND AND OBJECTIVES: Recent investigations indicated that continuous use of fertilizers and pesticides in agricultural fields not only deteriorated soil health but also caused a deleterious effect on surface and groundwater bodies. Treating such wastewater using microalgae has shown higher nutrient removal and biomass efficiency. Moreover, microalgae are proven to be miniature factories that augment the huge potential of biofuel. The aim of this study is to evaluate the different light intensities required for *Chlorella vulgaris* algae to remove nutrients from synthetic agricultural wastewater in a fabricated bubble column photobioreactor. Additionally, the research findings focus on assessing the degradation of organic pollutants and biomass generation under different light conditions.

METHODS: In this study, synthetic agrochemical wastewater was treated in a bubble column photobioreactor with blue, red, sunlight, and white light conditions. The treatment was conducted in a batch process with a hydraulic retention time of 21 days, using light intensity of 1800–2800 luminescence and a temperature maintained at 25–28° degrees Celsius.

FINDINGS: Under different lighting conditions, the blue light condition exhibited a higher biomass concentration of 3.99 gram per liter, with an estimated heat energy value of 1.278 kilojoule per liter. Moreover, in the blue light condition, scanning electron microscopy analysis showed no significant changes in the shape of *Chlorella vulgaris* and energy-dispersive X-ray analysis elemental composition exhibited the lowest oxygen-to-carbon ratio (1.03). Fourier transform infrared spectroscopy was used to illustrate the functional group of microalgae under different lighting conditions. The lipid, protein, carbohydrate, and amino acid contents were 3329–3332, 2116–2139, 1636–1645, and 545–662 per centimeter, respectively. The higher biomass potential from the wastewater treatment shows significant benefit in terms of feedstock and biofuel production.

CONCLUSION: The present investigation identified the nutrient reduction and biomass productivity to be more in blue light condition for *Chlorella vulgaris* algae. The investigation also assessed the potential of lipid, carbohydrate, and protein content in *Chlorella vulgaris*, which indirectly evaluates the biofuel potential of the species.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
43	9	4

*Corresponding Author:
Email: *sathyanr5@srmist.edu.in*
Phone: +984 081 2729
ORCID: 0000-0002-7446-4073

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INTRODUCTION

In recent years, energy has become a most valuable product, and many research studies are focused on generating sustainable energy for replacing fossil fuel. Conversely, recent investigations indicated that continuous use of fertilizers and pesticides in agricultural fields not only deteriorated soil health but also caused a deleterious effect on surface and groundwater bodies. Specifically, the nutrient-laden runoff from agricultural fields poses a great threat due to the excessive content of phosphate and nitrate, which are carried to natural water bodies (Díaz *et al*., 2012; Khalid *et al*., 2019). This leads to eutrophication in water bodies, causing an ecological imbalance that leads to water pollution. Integrated systems are found to be effective in treating agricultural wastewater and generating biomass, which produces value-added products such as biogas, biofertilizers, and biofuel. Bioenergy and bioeconomy from biomass have great scope to satisfy the need of energy demand in the future (Driver *et al*., 2014; Shahid *et al*., 2019). Compared with constructed wetland treatment, wastewater treatment using microalgae had shown the highest biomass efficiency with a pollution removal efficiency of 80–90 percent (%). Energy recovery from algal biomass has also become one of the sustainable ways for harvesting renewable energy processes (Cai *et al*., 2013; Hoang *et al*., 2022). Because of declining petroleum reserves, increasing fuel prices, and depleting natural resources, renewable energy has become an essential global factor. In recent years, microalgae's biofuel potential had attracted considerable commercial interest due to its carbon-neutral ecosystem and indigenous production (Kunjapur *et al*., 2010; Moshood *et al*., 2021). Microalgae that are capable of growing very rapidly can accumulate bioproducts, and they do not require either large quantities of freshwater or fertile land to grow. Hence the algae can be conveniently grown in municipal or industrial wastewater and can assist in bioremediation (Borowitzka, 1999; Cai *et al*., 2013). Microalgae also helps in tackling global warming by reducing atmospheric carbon dioxide (CO_2) and serves as an alternative animal feedstock due to its high harvesting index (Chen *et al*., 2011). It is also emphasized that algal biomass can be used as a supplement for proteins to animals as it contains a high protein concentration of 40%–70% (Amaral *et al*., 2020; Maryjoseph and Ketheesan, 2020). Microalgae had a great potential of lipid (fat) accumulation 1%–70% in their cell density and can convert waste organics into bioenergy (H. Kamyab *et al*., 2017). Photoautotrophic microalgal growth depends on light intensity, CO_2, temperature, and nutrient availability in the photosynthesis process (Martínez Sancho *et al*., 1999). Photobioreactors (PBRs) are enclosed systems that help in the growth of photoautotrophic organisms to treat wastewater without any external containment with the aid of an artificial light source to facilitate photosynthesis (Pulz and Scheibenbogen, 2007). PBRs are of two types: a) an open PBR normally a raceway pond and b) closed PBR, which includes a bubble column, tubular flat plate, and spiral (Acién *et al*., 2017; de Vree *et al*., 2015). Considering the several limitations of open PBR, closed PBR is often preferred for bioremediation. Among different closed PBRs, bubble column reactors are generally preferred because of their simple design and construction. Besides, these reactors consume less floor space, are less prone to contamination, and are efficient in CO_2 utilization (Chinnasamy *et al*., 2010; Gupta, Lee, and Choi, 2015). Because light is an energy source that serves as an environmental factor for developing photosynthetic organisms in bubble column PBRs (BC-PBRs), it can be provided either naturally or artificially using lamps (Pulz and Scheibenbogen, 2007). The use of sunlight as a light source for microalgae is advantageous as it is free, cost effective, and abundant, but its temperature should be considered for microalgal growth systems (Xin *et al*., 2011). However, the duration of day and night periods, changing weather and climatic conditions, nonuniform light intensity, and other seasonal changes may influence the efficiency of the reactor (Singh and Singh, 2015). These drawbacks can be avoided by installing artificial lighting systems with continuous or intermittent illumination in PBRs to enhance biomass productivity (Jung *et al*., 2019). The biofuel ability of certain microalgae can be enhanced by modifying the artificial light supply and factors such as culture conditions, nitrogen depletion, and temperature. Several studies investigated the effects of light, temperature, and CO_2 on the growth of microalgae, such as *Scenedesmus*, *Spirulina platensis*, *Dunaliella salina*, *Nannochloropsis oceanica*, and mixed cultures of the *Chlorella* species (Masojídek and Torzillo, 2014; Mohsenpour *et al*., 2021; Xin *et al*., 2010, 2011). Generally, light-emitting diodes

(LEDs) are more frequently used than traditional light sources for microalgae cultivation. LEDs consume less power and are energy efficient. They are characterized by their long lifespan, less heat or no ultra voilet (UV) emissions, and instant lighting condition. They are also highly reliable in case of system-level failures. Because the absorption bands are seen in blue and red spectral areas of the chlorophyll molecule, LEDs are considered a good source compared with fluorescent light due to their broad visible spectrum. Indeed, microalgae require optimal irradiation conditions with narrow bands of light to maximize their photosynthetic rates, which can be achieved using LEDs (Borella *et al*., 2022). Because microalgae are a photoautotrophic organism, the effects of light, such as white, blue, and red light and sunlight conditions: white light conditions (WC), blue light conditions (BC), red light conditions (RC), and sunlight conditions (SC), respectively on microalgae are investigated to understand its influence in biomass production. The experiments were conducted in BC-PBRs to understand the optimum light intensities and favorable conditions required for the algae for nutrient removal, degradation of organic pollutant, and biomass generation. This study aims to treat high wastewater nutrients using *Chlorella vulgaris* in a BC-PBR and evaluate the effect of different light intensities required for *C. vulgaris* algae to remove nutrients from synthetic agricultural wastewater, assess nutrient pollutant degradation, and determine biomass generation. This study has been conducted in the Environmental Engineering Laboratory, Department of Civil Engineering, SRM Institute of Science and Technology, Kattankulathur, India, in 2022.

MATERIALS AND METHODS

Microalgae strains and culture conditions

The microalgal strain *C. vulgaris* was considered for this study. The algal strain was obtained from the Annakili Algal Research Institute, Chennai, India. Fig. 1 shows the algal strain culture under 20- and 10-micrometer (µm) magnifications obtained using the Hover labs Trinocular Research Coaxial Microscope. The algae were precultivated using culture medium with the following operating conditions: white LEDs with a light intensity of 1300–1800 luminescence (lux) were used under a light-to-dark period of 16:8. The optimum room temperature varied from 23°C ± 2°C (degrees Celsius) (Tripathy and Kumar, 2022). Bold's Basal medium (BBM) was used to cultivate the strains of microalgae that were then centrifuged (Remi R-8C, India) at 1,957 × *g* for 5 min before being rinsed in deionized water and centrifuged again for 5 min. The suspended microalgae were collected and inoculated in BBM. Later, the cultured microalgae strains were introduced into the BC-PBRs with a 10-milliliter per liter (mL/L) dilution (Sevugamoorthy and Rangarajan, 2023).

Synthetic agrochemical wastewater

Synthetic agrochemical wastewater (SACWW) was prepared by slightly modifying the compositions of ammonium dihydrogen phosphate ($[NH_4]$ H_2PO_4) and phosphorus pentoxide (P_2O_5). Because SACWW acts as a source of nutrients for microalgal cells,

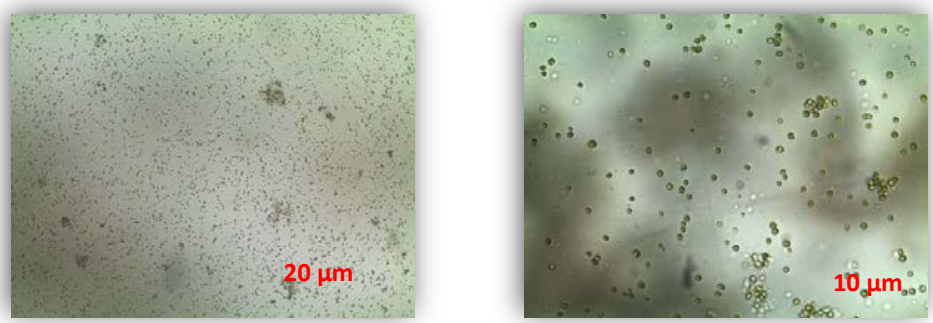


Fig. 1: Light microscopic images of *C. vulgaris* under (a) 20-µm and (b) 10-µm magnifications

high quantities of ammonium and phosphorus were obtained as per the American Public Health Association (APHA) method in BC-PBRs (Martínez Sancho *et al.,* 1999). Glucose (carbon source) and chemicals such as ammonium chloride (NH_4Cl), sodium nitrate ($NaNO_3$), sodium chloride (NaCl), copper sulfate pentahydrate ($CuSO_4.5H_2O$), and cobalt(II) nitrate hexahydrate ($CO[NO_3]_2.6H_2O$) were also added in BC-PBRs with pH kept near 7. The initial characterization of the wastewater for pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonium, and phosphorus were 6.8, 1900, 6500, 90, and 23 mg/L, respectively (Anusha Gowri *et al*., 2022).

Experimental setup and reactor conditions

For the experimental setup, four BC-PBRs with 190-millimeter (mm) inner diameter, 5-mm thickness, and 500-mm depth were made with a transparent acrylic sheet. The reactor had a total volume of 20L. During the treatment process, a liquid and gas volume of 12 and 8 liter (L), respectively, was maintained at a temperature of 23°C ± 2°C in laboratory conditions to ensure uniform luminescence throughout the research period. From previous studies, it is learned that the support system of cylindrical or tubular BC-PBRs occupied more land space (Díaz, Inostroza, and Acién Fernández 2019; Camacho *et al*., 2011), and to overcome this, BC-PBRs were designed to stand on the ground without any external support (Fig. 2). The reactors were exposed to cycles of 16 h of light followed by 8 h of darkness, using external different-colored LED lights emitting BC, RC, and WC; lights providing a medium illuminance of approximately 2 kiloluminescence (klux); and a natural SC. The cultures in BBM were grown in 2-L Erlenmeyer under ideal SC for a photoperiod of 16:8-h light-to-dark phases. The initial cell concentration of microalgae for all lighting conditions in BC-PBR was adopted in the ratio of 1:10 (100 mL of cultured microalgae to 1 L of synthesized wastewater). An air diffuser motor supplying 3-L/min rate of air was used in PBR to prevent the accumulation of algae at the bottom and to ensure complete light supply to the microalgae inside the reactor. (Fig. 2) shows the experimental setup of *C. vulgaris* under different light conditions.

Lighting conditions and its configurations

Light is one of the most important factors in PBR. The light intensity, photoperiod, and light wavelength also constitute vital elements in PBRs (Czeczuga, 1986). LED bulbs were considered for this study. One of the major advantages of LED was its low energy consumption and in providing high illumination. These bulbs are readily available in the market and are 40%–60% cheaper than compact fluorescent light bulbs (Janssen *et al*., 2000; Ra *et al*., 2016; Borella *et al*., 2022). LED lights for three BC-PBRs arranged with 20 diodes spaced at 2-cm intervals and one BC-PBR setup at sunlight were used as the light source for photoperiodic effect for growth of the microalgae for this study (Fig. 2) and their labeling is discussed in (Table 1). LED strips emitting white 450-, blue 465-, and red 660-nanometer (nm) light were evaluated for the effects on algal growth rate with a 12-h light and 12-h dark photoperiod (de Mooij *et al*., 2016; Pulz and Scheibenbogen, 2007; Silva *et al*., 2022).

Analytical techniques

APHA 2012 was used for monitoring the physiochemical parameters, namely, pH, organic pollutants, and nutrients like ammonium and phosphorus concentrations, before and after the treatment. A pH probe (SYSTRONICS, India) was used to monitor the pH variations in the sample. COD and BOD were evaluated using the open reflux method and Winkler's method, respectively. UV spectroscopy operating at 620 and 420 nm was used to monitor phosphorus and ammonium, respectively (Shimadzu UV-VIS 1900i, Japan) (APHA 2012 "WPCF 2012).

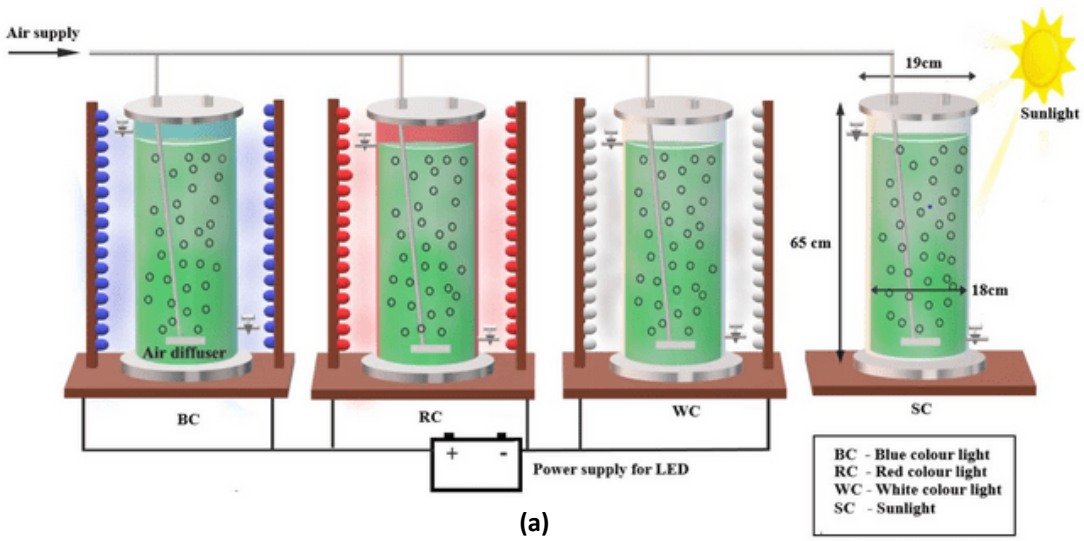
Growth estimation

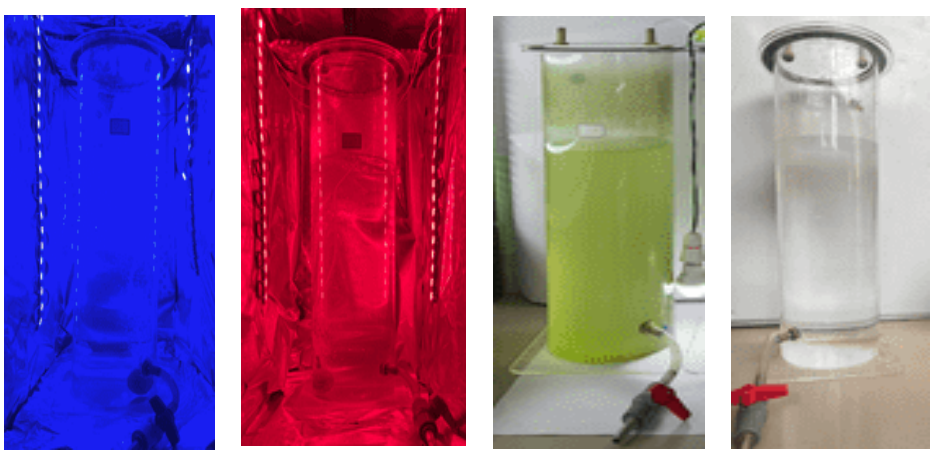
The growth pattern of the inoculated culture was determined using the optical density (OD) at 680 nm (Shimadzu UV-VIS 1900i, Japan) daily for 24 days until the culture reaches its stationary phase.

Biomass analysis

A Whatman filter (GE Healthcare Lifesciences, Grade 4) with a diameter of 125 mm was used to filter the microalgal biomass that had grown in wastewater. The empty oven dry filter paper weight (W_1) and drying the algal biomass at 105°C for 24 h, the dry weight of the filter containing algal biomass (W_2) were measured. The microalgal concentration (mg/L) was obtained using Eq. 1 (Pruvost *et al*., 2009).

$$\text{Biomass analysis (mg/L)} = (W_2 - W_1)/V \quad (1)$$





(b)

Fig. 2: (a) Pilot scale bubble column photobioreactor (BC-PBR) model for microalgal growth with different lighting conditions and (b) photographic view of BC-PBRs

Where, W_1 is the empty oven dry weight of filter paper, W_2 is the dry algal weight of filter paper (after oven drying at 105°C), and V is the volume of wastewater after the treatment.

Nutrient and organic pollutant removal

The nutrient removal efficiency of ammonium and phosphorus was calculated in alternate days of sampling, and organic pollutant removal was identified by influent and effluent sampling based on Eq. 2 (Pruvost *et al*., 2009).

Nutrient and organic pollutant = (Initial concentration − Final concentration)/(Initial concentration) (2)

where the initial concentration is the influent wastewater before treatment in BC-PBRs and the final concentration is the effluent wastewater from the reactor after the treatment in BC-PBRs.

Scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) analyses

SEM and EDX analysis images were considered to

Table 1: Lighting parameters and their intensities

Symbol	Lighting conditions	Temperature (°C)	Wavelength (nm)	Light intensity (klx)	pH
BC	Blue light	24 ± 2	400–500	1.8–2.8	6.9–7.2
RC	Red light	24 ± 2	600–700	1.8–2.8	6.9–7.2
WC	White light	24 ± 2	300–400	1.8–2.8	6.9–7.2
SC	Sunlight	28 ± 4	520–700	1.8–4.8	6.9–7.2

illustrate the algal cell morphology under treated and untreated conditions. Algal cells were treated with 2.5% glutaraldehyde and dehydrated using ethanol (30%–100% concentration). The cells were then dried in a hot air oven, mounted on protective film in the molds, and sputtered with chromium. SEM micrographs of algal cells and their elemental composition were captured using Thermo Fisher Apreo S, USA.

Differential scanning calorimetry (DSC) analysis for thermal properties of microalgae

For the DSC analysis, microalgae from the late exponential phase were harvested from the BC-PBRs and were centrifuged using REMI R8C at a rate of 5000 revolutions per minute for 15 min. Later, the biomass from the instrument was collected and washed twice with deionized water and then dried in a hot air oven at 80°C for a day. The dried biomass was then pulverized using porcelain mortar and stored in a desiccator. The stored biomass was then assessed for its combustion property in a Differential Scanning Calorimeter (NETZSCH, Germany) under 10°C/min and 30°C/min with nitrogen gas supply. The correlation between weight loss and its respective temperature was recorded continuously, and the DSC plot was established to determine the calorimetric value of the algal biomass to understand its bioenergy potential.

Fourier transform infrared (FTIR) spectroscopy *measurements*

The FTIR spectra of all algal consortia were investigated to identify the shifts in different functional groups. FTIR spectra were obtained at ambient temperature, using a (Bruker Alpha, Germany), and the FTIR spectrometer wavelength ranges between 500 and 4000 /cm.

RESULTS AND DISCUSSION

Comparison of OD values in different light conditions

The colorimetric method was used for analyzing OD with the range of 680 nm. SACWW was introduced in BC-PBR and was operated in batch mode until it reached the stationary phase. The growth ability of microalgae was monitored under different lighting conditions (Fig. 3) because it enhances the growth cell structure along with nutrients. A comparison of OD values between different light conditions exhibits the growth variation in SACWW. Under BC and RC, OD at 680 nmshows a similar result by achieving a maximum value of 0.34 on the 18th day of treatment, which indicates the higher BC and RC wavelengths of 500 and 600 nm, respectively, with a uniform average intensity of 2 klx. Followed by that, SC showed 0.33 on the 19th day of treatment, although the intensity varied from time to time due to the availability of daylight conditions. WC showed a lesser value of 0.24 on the 17th day of the treatment process. The OD results clearly showed that the algal cells placed in different lighting conditions achieved their exponential growth between the 10th and 17th days.

Comparison of algal biomass in different light conditions

Biomass productivity is one of the major benefits of PBRs. A comparison of algal biomass in different lighting conditions is shown in Fig. 4. The biomass productivity of 4.15 g/L at the end of 24 days obtained in SC was better than that in other lighting conditions. BC produced a biomass of 3.99 g/L, followed by both RC and WC with biomass of 2.55 g/L. Although the experimentation was limited to 24 days, the microalgal growth rate was also found to be in its declining phase after this period. The results confirmed the biomass potential of *C. vulgaris* under all lighting conditions.

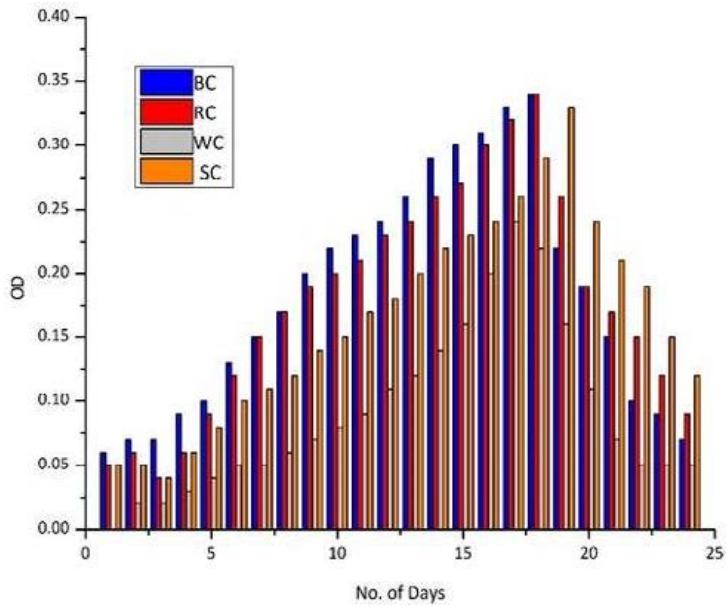


Fig. 3: The OD values of *C. vulgaris* under different light conditions

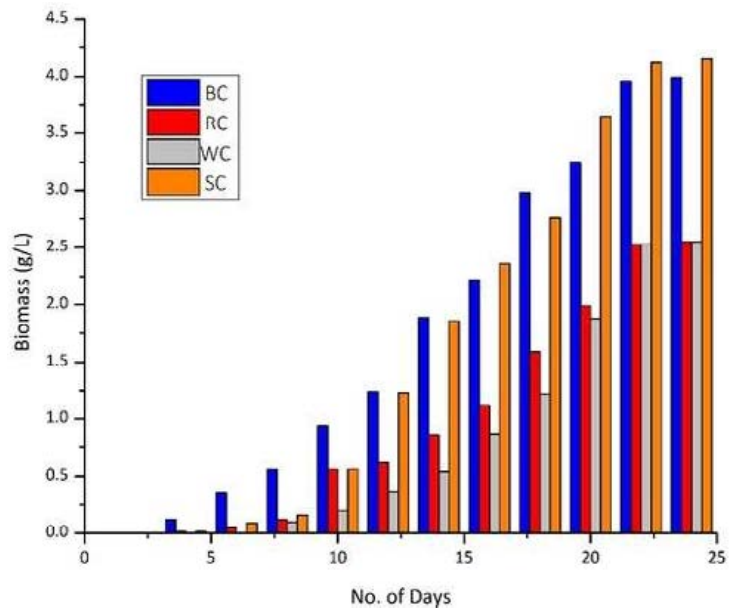


Fig. 4: Biomass concentration of *C. vulgaris* in different lighting conditions

Comparison of nutrient removal under different light conditions

Fig. 5 depicts the significant reduction in the ammoniacal nitrogen concentration in all four lighting conditions. In the first 12 days of exposure, the nutrients present in the wastewater were removed quite similarly in all lighting conditions. The NH_4–N concentration on the 12th day in the BC was reduced from 90 to 14 mg/L, signifying an 85% nutrient reduction in the initial growth phase of microalgae.

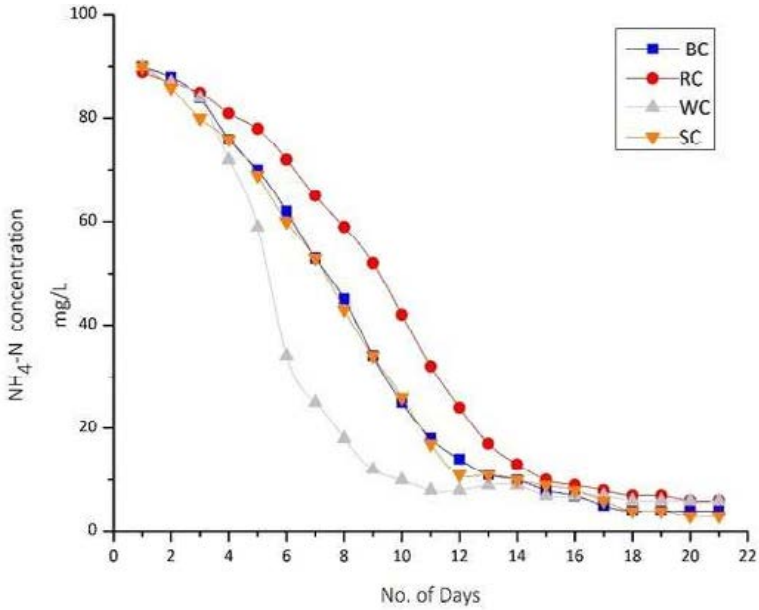


Fig. 5: NH_4–N removal of *C. vulgaris* in different lighting conditions

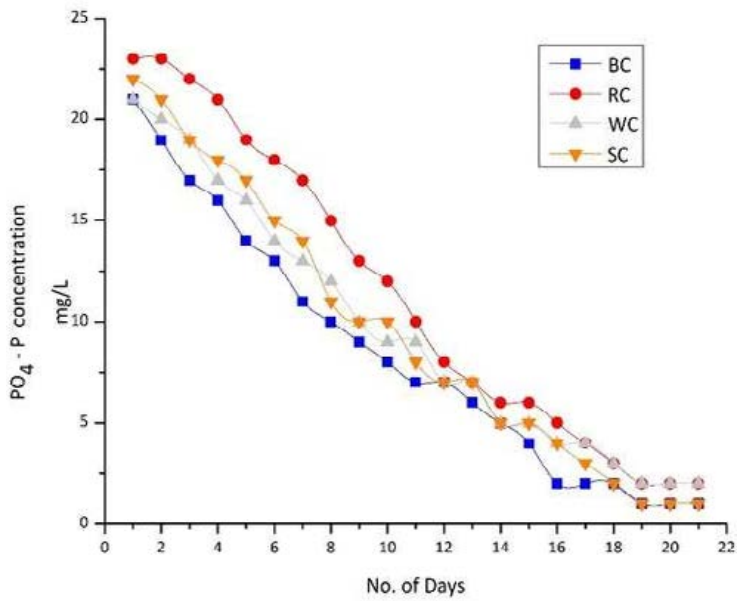


Fig. 6: PO_4–P removal of *C.* vulgaris in different lighting conditions

The overall NH_4–N removal rate was found to be 95.5% in the BC. In SC, the nutrient reduction was 87% at the end of the 12th day with an overall removal rate of 96.6%. RC and WC showed 73% and 91% nutrient reduction on the 12th day, respectively. In all lighting conditions, *C. vulgaris* showed significant NH_4–N removal and is found to be significantly high at ~93% from treating SACWW in BC-PBR with a retention time of 24 days.

Fig. 6 shows the variation of PO_4–P in different lighting conditions for the retention period of 24 days. The nutrients present in wastewater

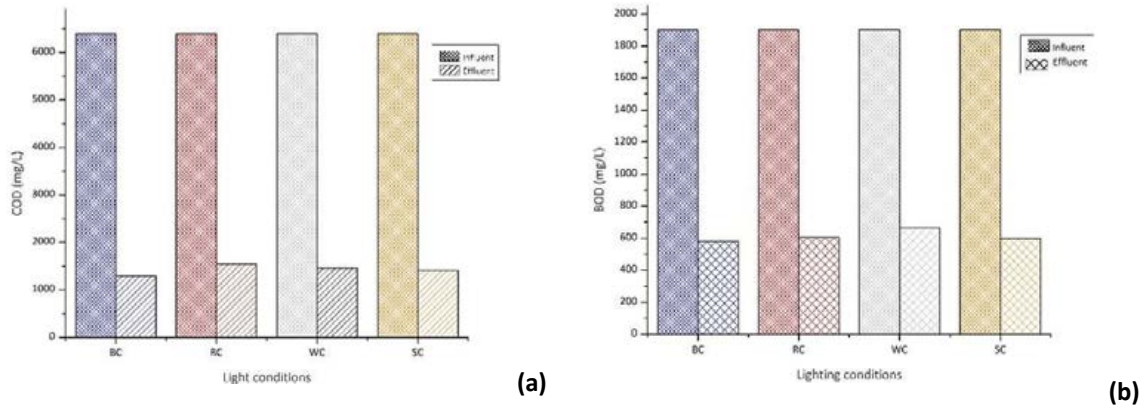


Fig. 7: (a) COD removal rate of *C. vulgaris* in different light conditions and (b) BOD removal rate of *C. vulgaris* in different light conditions of BC-PBRs

Table 2: Comparison of SACWW using *C. vulgaris* in BC-PBRs under different light conditions

Light condition	Initial concentration (before treatment (mg/L)				Final concentration (after treatment (mg/L)				Reduction (%)			
	NH_4–N	PO_4–P	COD	BOD	NH_4–N	PO_4–P	COD	BOD	NH_4–N	PO_4–P	COD	BOD
BC	90	21	6400	1880	4	1	1210	578	95.5	95.2	81	69
RC	89	23	6394	1990	5	2	1340	603	93.2	91.3	79	65
WC	90	21	6389	1910	5	2	1415	665	93.3	90.4	77	68
SC	90	22	6397	1900	3	1	1329	597	96.6	95.4	79	68

were significantly removed in the first 14 days of exposure under different lighting conditions, and the overall removal rate was approximately 90% for the retention period. At the end of 14 days, the PO_4–P concentration was reduced from 21 to 5 mg/L (i.e., 76%) with an overall removal rate of 90% under BC. The nutrient reduction at the end of 14 days and the overall removal rate were found to be 77% and 90%, 73% and 91%, and 76% and 90% in SC, RC, and WC, respectively.

Comparison of COD and BOD removal efficiency under different lighting conditions in BC-PBRs

The COD and BOD represents the organic matter found in the wastewater. The air supplied inside the reactor not only enhances the mixing regime but also initiates the oxidation of organic compounds inside the reactor (Ting *et al.*, 2017). Besides, CO_2 fixation and O_2 transformation during biodegradation indirectly enhance the degradation of organic matter in the reactor. Before and after the treatment, the COD removal rate in BC-PBRs under different light conditions was analyzed (Fig. 7a). BC exhibited 81% COD removal, followed by SC with 79%. The COD removal rate was 78% for both RC and WC. The BOD in BC-PBRs analyzed before and after the treatment under different light conditions is depicted in Fig. 7b. The maximum BOD removal rate of 70% was exhibited by BC. The BOD removal rate in SC, RC, and WC were 69%, 68%, and 65%, respectively.

Comparison of pollutant removal before and after treatment under different light conditions using BC-PBRs

Table 2 shows the pollutant removal before and after the treatment of SACWW using *C. vulgaris* under different light conditions. As per the Environmental Protection Rules (1986) standard of India, the concentration of ammoniacal nitrogen should be 50 mg/L, dissolved phosphate should be <5 mg/L, and COD and BOD should be <250 and 30 mg/L, respectively. Although COD and BOD are slightly higher than the stipulated standard, it is required to be treated before being discharged in freshwater bodies. Although microalgae are an efficient medium

for treating nutrient pollutants, they have limitations in COD and BOD removal. In general, primary and secondary treatment plants remove 60%–80% of COD and BOD, and when the effluent reaches the tertiary PBR units using microalgae, the remaining organic pollutants will be treated.

Comparison of SEM and EDX analyses under different light conditions

SEM analysis was performed to investigate the variations in surface morphologies of *C. vulgaris* strains before and after the treatment of SACWW under different lighting conditions in BC-PBRs. To investigate the changes in the algal strain after the treatment, SEM images were obtained in different magnifications. Fig. 8 shows SEM and EDX analyses of *C. vulgaris* under BC, RC, WC, and SC after wastewater treatment with 20- and 5-µm magnifications. It is observed that under BC and SC, the algal strains exhibited distinct shapes, rigid cell walls, and

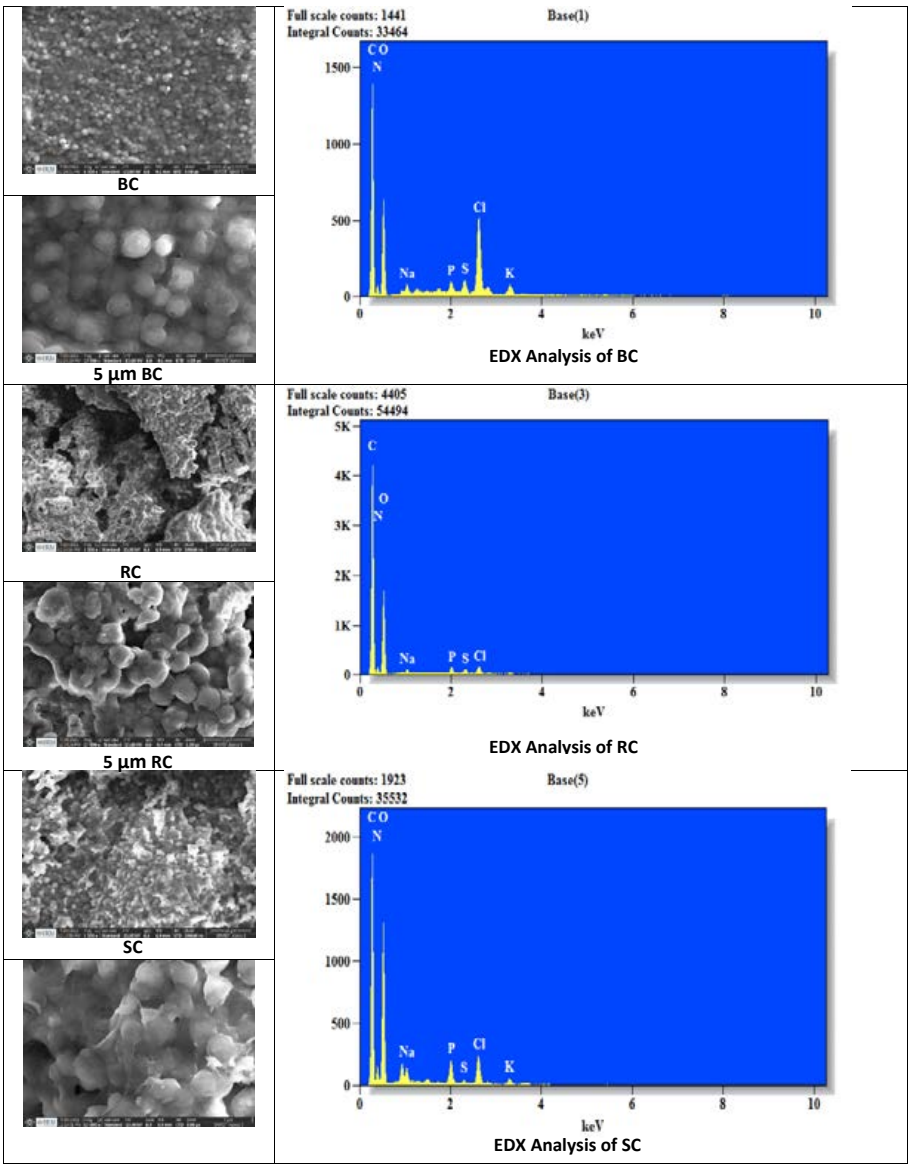
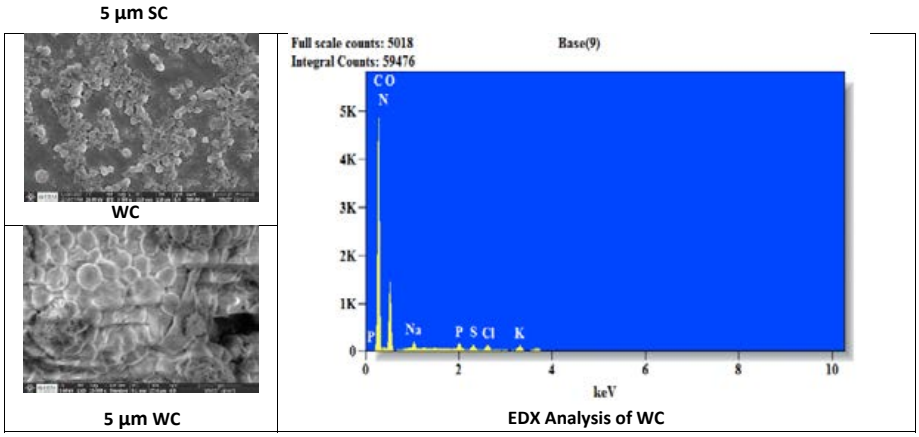


Fig. 8: SEM images of BC, RC, WC, and SC under 20- and 5-µm magnification and EDX analyses of BC, RC, SC, and WC



Continued Fig. 8: SEM images of BC, RC, WC, and SC under 20- and 5-µm magnification and EDX analyses of BC, RC, SC, and WC

smoothness, which were comparatively equal to those of algal cells in a culture condition. Conversely, algal strains under RC and WC displayed a nondistinct shape with slightly damaged cell walls after the treatment, signifying their stressed condition.

Elemental analysis of C. vulgaris *under different lighting conditions using SEM-EDX*

The organic elements for *C. vulgaris* under different light conditions are shown in Fig. 8, which mainly contains carbon (C), oxygen (O), and nitrogen (N). Other insignificant elements such as sodium (Na), chlorine (Cl), phosphorus (P), potassium (K) were also identified. Table 3 shows the percentage weight of elements harvested under different light conditions: the elements C, O, and N ranges between 37.33 and 28.17, 48.23 and 35.51, and 15.85 and 14.71, respectively. In BC, the percentage atomic weight of C, O, and atomic oxygen-to-carbon (O/C) ratio is 34.44%, 43.54%, and 1.03%, respectively, which is slightly higher than the O/C value exhibited by terrestrial crops such as sugarcane bagasse (0.88), corn (0.8), and pine waste (0.88) (Hossain *et al*., 2019). The lowest O/C ratio (1.03%) is achieved in BC, which is equal to the biomass of rice husk (Bousdira *et al*., 2014). Phukan *et al*. (2011) documented that O/C ratios were directly associated with the energy content of solid fuel.

DSC analysis of biomass under different light conditions

DSC analysis under different light conditions is shown in Table 3. In a heating cycle, the microalgae exhibits peak stretches, which indicate the decomposition process, with its respective enthalpies. The harvested biomass from BC-PBRs is evaluated for its energy potential, and the exothermic events with enthalphy (ΔH) are shown in Table 4. The peak ΔH was observed as 308.2 J/g at 115.6°C, followed by a second exothermic event of 50.96 J/g at transition temperature of 197.1°C under RC. But the biomass generation was high under BC, which is shown in Fig. 4. Overall, BC showed a high estimated enthalpy of 1.278 kilojoule per liter (kJ/L). Although algal biomass has bioenergy potential, the results infer that light conditions will have a significant effect on their nature.

FTIR spectroscopy of C. vulgaris *under different lighting conditions*

FTIR spectroscopy assessed the properties of *C. vulgaris* before and after treatment under different light conditions with various characteristic functional groups in the range of 4000–450/cm, as shown in Fig. 9. FTIR spectra indicated the presence of lipid, protein, carbohydrate, and amino acid stretching vibration peaks in cells at 2800–3300, 1700–2800, 1500–1700, and 700–500/cm, respectively. The determination of lipid, carbohydrate, and protein content using FTIR has been well documented by many researchers (Sharma *et al*., 2018, 2019). In a previous study, Sharma *et al*. (2018, 2019) documented the characteristic peaks of O–H, C–H, C≡C, C=O, and N–H amide after wastewater

Table 3: Elemental analysis of biomass from BC-PBRs under different light conditions using SEM-EDX

Elements	Light conditions	Weight (%)	Atomic (%)
Carbon (C)	BC	34.44	43.54
	RC	34.88	41.53
	SC	28.17	35
	WC	37.33	44.60
Oxygen (O)	BC	35.51	33.70
	RC	45.34	40.52
	SC	48.23	44.98
	WC	41.22	36.98
Nitrogen (N)	BC	14.71	15.95
	RC	15.85	16.19
	SC	15.30	16.30
	WC	15.26	15.63
Chlorine (Cl)	BC	10.22	4.38
	RC	1.79	0.72
	SC	3.74	1.58
	WC	1.16	0.47
Sodium (Na)	BC	0.67	0.44
	RC	0.42	0.26
	SC	0.51	0.33
	WC	1.14	0.71
Phosphorus (P)	BC	1.22	0.60
	RC	0.95	0.44
	SC	2.27	1.09
	WC	1.15	0.53
Sulfur (S)	BC	1.55	0.73
	RC	0.78	0.35
	SC	0.40	0.19
	WC	0.86	0.39
Potassium (K)	BC	1.69	0.66
	RC	-	-
	SC	1.38	0.53
	WC	1.88	0.69
O/C (%)	BC	1.03	0.77
	RC	1.30	0.97
	WC	1.71	1.28
	SC	1.10	0.82

Table 4: DSC analysis of biomass from BC-PBRs after treatment under different light conditions

Light conditions	ΔH (J/g)			Temperature (°C)		Biomass generated (g/L)	Estimated ΔH (kJ/L)
	1st peak	2nd peak	Total	1st peak	2nd peak		
BC	283.4	37.02	320.42	112.2	194.8	3.99	1.278
RC	308.2	50.96	358.98	115.6	197.1	2.55	0.915
SC	252.7	46.44	299.47	105.4	198.9	4.15	1.242
WC	278.4	-	278.4	105.4		2.55	0.71

treatment to be 3300, 2900, 2100, 1600, and 600/cm, respectively. In this study, after treating SACWW with different light conditions, the exhibited peak of the O–H, C≡C, C=O, and N–H functional groups was in the range of 3329–3332, 2116–2139, 1636–1645, and 545–662/cm, respectively (Miglio *et al.*, 2013).

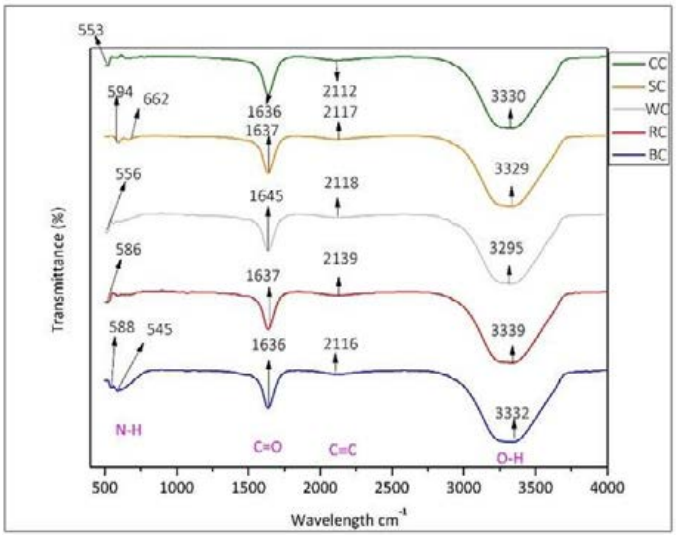


Fig. 9: FTIR spectroscopy of *C.* vulgaris under different light conditions after SACWW treatment

CONCLUSIONS

In this study, *C. vulgaris*, a blue–green microalgal species that have a tendency to treat wastewater, were cultivated under different light conditions, namely, BC, RC, SC, and WC, in BC-PBRs to treat SACWW. Among the four different lighting conditions, *C. vulgaris* under BC was found to be the most efficient for treating synthesized agricultural wastewater. The algae exhibited the highest growth rate under BC (400–500 nm), followed by SC (520–700 nm), with more effective treatment processes compared with those of RC (600–700 nm) and WC (300–400 nm). OD values clearly showed the exponential growth of algae falls between the 10th and 17th day irrespective of the lighting conditions. SC (91%) showed the highest COD removal rate, followed by BC, RC, and WC with a removal rate of 81% and 78%, respectively. In BOD removal, BC and SC showed similar degradation (~70%), followed by RC (68%) and WC (65%). The maximum ammonium nitrate (NH_4–N) and phosphorus (PO_4–P) removal rates were found in SC (96.6% and 91%, respectively), followed by BC (95.5% and 90%, respectively), RC (73% and76%, respectively), and WC (91% and 90%, respectively). SEM and EDX analyses in SC and BC indicated a more rigid cell structure and higher O/C ratio after SACWW treatment. FTIR spectroscopy of algal consortia helps to understand the biochemical functional group of microalgae before and after treatment, revealing their potential for biofuel production. Vibration peaks of lipids, proteins, carbohydrates, and amino acids in cells were 2800–3300, 1700–2800, 1500–1700, and 700–500/cm, respectively. The application of LEDs in algal culturing has become quite common. This study investigated the biomass growth under different-colored LEDs and demonstrated the bioenergy potential of algal biomass as a resource for biofuel production due to the high lipid, carbohydrate, and protein content in *C. vulgaris.* The biomass produced from the algae can be converted to biofuel, such as bioethenol and biobutanol, which has a huge potential to convert light energy into sustainable bioenergy through wastewater treatment. Although nutrient removal is the primary focus of the research work, the generation of algal biomass as a supplementary by-product enhances the biofuel capability of the algae.

AUTHOR CONTRIBUTIONS

S. Dhanasekar, the first author, has contributed in conceptulization, experimentation, data analysis, manuscript preparation and interpolation of results. R. Sathyanathan corresponding and second authour has contributed supervising and revising the manuscript.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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PUBLISHER'S NOTE

ABBREVIATIONS

%	Percent
°	Degree
-	Single bond
=	Double bond
≡	Triple bond
°C	Degree Celsius
°C/min	Degree Celcius per minute
ΔH	Enthalpy
~	Approximately equal
Abs	Absorbance
APHA	American public health association
BBM	Bold's Basal Medium
BC	Blue light conditions
BC-PBRs	Bubble column photobioreactors
BOD	Biochemical oxygen demand
C	Carbon
C. vulgaris	Chlorella vulgaris
Cl	Chlorine
/cm	Per Centimeter
cm	centimeter
CO_2	Carbondioxide
COD	Chemical oxygen demand
d	Day(s)
DSC	Differential Scanning Calorimetery
e.g	Exempli gratia (for example)
EDX	Energy dispersive X-ray analysis
eq	Equation
FTIR	Fourier transfrom infra red spectroscopy
g	Gram (s)
g/L	Gram(s) per liter
H	Hydrogen
i.e.	Id est (that is)
K	Pottasium
J/g	Joules per gram
kJ/L	Kilojoule per liter
Klux	KiloLuminescence
km	Kilometer
L	Liter
L/D	Light/Dark
LED	Light emmiting diodes
lx	Luminescence
m	Meter
m^2	Meter square
mg/g	Miligram per gram
mg/L	Milligram per liter

mL/L	milliliter per liter
mm	Millimeter
mm/g	Millimeter per gram
min	Minute
N	Nitrogen
Na	Sodium
$NH_4H_2PO_4$	Ammonium dihydrogen phosphate
NH_4-*N*	Ammonium nitrate
nm	Nanometer
No.	Number
O	Oxygen
O/C	Atomic oxygen-to-carbon ratio
OD	Optical density
P	Phosphorous
pH	Potential of hydrogen
P_2O_5.	Phosphorus pentoxide
PBRs	Photobioreactors
PO_4-*P*	Phosphate
RC	Red light conditions
rpm	Revolution per minute
S	Sulphur
SACWW	Synthetic agrochemical wastewater
SC	Sunlight conditions
SEM	Scanning electron microscopy
UV	Ultra voilet
UV–Vis	Ultravoilet-Visible spectroscopy
V	Volume
Viz	Videlicet
W	Weight
WC	White light conditions
µm	Micrometer

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AUTHOR (S) BIOSKETCHES

Dhanasekar, S., Ph.D. Candidate, Assistant Professor, Department of Civil Engineering, Faculty of Engineering and Technology, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu, Tamil Nadu 603203, India.
- Email: *dhanases@srmist.edu.in*
- ORCID: 0000-0001-9465-028X
- Web of Science ResearcherID: GNH-2305-2022
- Scopus Author ID: 5710701824
- Homepage: https://www.srmist.edu.in/faculty/mr-dhanasekar-s/

Sathyanathan, R., Ph.D., Associate Professor, Department of Civil Engineering, Faculty of Engineering and Technology, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu, Tamil Nadu 603203, India.
- Email: *sathyanr5@srmist.edu.in*
- ORCID: 0000-0002-7446-4073
- Web of Science ResearcherID: ABE-8647-2021
- Scopus Author ID: 56365696100
- Homepage: https://www.srmist.edu.in/faculty/dr-r-sathyanathan-2/

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